

TABLE 5

BIOASSAY OF CRY1C LOOP α 3-4 MUTANTS USING *SPODOPTERA EXIGUA* LARVAE

Strain	Toxin	LC ₅₀ ¹ (95% C. I.) ³	LC ₉₅ ² (95% C. I.)
EG11726	Cry1C	116 (104-131)	1601 (1253-2131)
EG11740	Cry1C.563	50 (42-59)	583 (433-844)
EG11747	Cry1C.499	67 (58-78)	596 (455-834)
EG11746	Cry1C.579	68 (58-79)	554 (427-766)

¹ Concentration of Cry1C protein that causes 50% mortality expressed in ng crystal protein per 175 mm² well. Results of 3-7 sets of replicated bioassays.

² Concentration of Cry1C protein that causes 95% mortality expressed in ng crystal protein per 175 mm² well. Results of 3-7 sets of replicated bioassays.

³ 95% confidence intervals.

TABLE 6

BIOASSAYS USING *PLUTELLA XYLOSTELLA* LARVAE

Strain	Toxin	LC ₅₀ ¹ (95% C. I.) ³	LC ₉₅ ² (95% C. I.)
EG11726	Cry1C	92 (83-102)	444 (371-549)
EG11740	Cry1C.563	106 (95-119)	579 (478-728)
EG11811	Cry1C R148A	61 (45-85)	400 (241-908)

¹ Concentration of Cry1C protein that causes 50% mortality expressed in ng crystal protein per 175 mm² well. Results of two sets of replicated bioassays.

² Concentration of Cry1C protein that causes 95% mortality expressed in ng crystal protein per 175 mm² well. Results of two sets of replicated bioassays.

³ 95% confidence intervals.

The Cry1C mutant strains EG11811 (Cry1C R148A) and EG11815 (Cry1C R180A) were grown in C2 medium and evaluated using the same quantitative eight-dose bioassay procedure. The insecticidal activities of Cry1C and Cry1C R180A against *S. exigua* and *P. xylostella* were not significantly different, however, Cry1C R148A exhibited a 3.6-fold lower LC₅₀ and a 3.7-fold lower LC₉₅ against *S. exigua* when

compared to the original Cry1C-endotoxin (Table 7). Cry1C R148A and Cry1C exhibited comparable insecticidal activity against *P. xylostella* (Table 6).

TABLE 7
BIOASSAYS OF CRY1C R148A USING *SPODOPTERA EXIGUA* LARVAE

Strain	Toxin	LC ₅₀ ¹ (95% C. I.) ³	LC ₉₅ ² (95% C. I.)
EG11726	Cry1C	141 (122-164)	1747 (1279-2563)
EG11811	Cry1C R148A	41 (33-52)	481 (314-864)

¹ Concentration of Cry1C protein that causes 50% mortality expressed in ng crystal protein per 175 mm² well. Results of two sets of replicated bioassays.

² Concentration of Cry1C protein that causes 95% mortality expressed in ng crystal protein per 175 mm² well. Results of two sets of replicated bioassays.

³ 95% confidence intervals.

The Cry1C mutant strains EG11811 (Cry1C R148A), EG11740 (Cry1C.563), and EG11726 (producing wildtype Cry1C) were similarly cultured and evaluated in bioassays using neonate larvae of *Trichoplusia ni*. The insecticidal activities of Cry1C R148A and Cry1C .563 against *T. ni* exhibited a lower LC₅₀ and LC₉₅ against *T. ni* when compared to EG11726 (Table 8).

TABLE 8
BIOASSAYS USING *TRICHOPLUSIA NI* LARVAE

Strain	Toxin	LC ₅₀ ¹	LC ₉₅ ²
EG11726	Cry1C	40 (31-56) ³	330
EG11740	Cry1C.563	20 (17-24)	104
EG11811	Cry1C-R148A	19 (16-23)	115

¹ Concentration of Cry1C protein that causes 50% mortality expressed in ng crystal protein per 175 mm² well. Results of one set of replicated bioassays.

² Concentration of Cry1C protein that causes 95% mortality expressed in ng crystal protein per 175 mm² well. Results of one set of replicated bioassays.

³ 95% confidence intervals.

Bioassay comparisons with other lepidopteran insects revealed additional improvements in the properties of Cry1C.563 and Cry1C-R148A, particularly in toxicity towards the fall armyworm *Spodoptera frugiperda* (Table 9) The doses reported in Table 8 are as follows: 10,000 ng/well *A. ipsilon*, *H. virescens*, *H. zea*, *O. nubilalis*, and *S. frugiperda*.

TABLE 9
BIOASSAY COMPARISONS WITH OTHER LEPIDOPTERAN INSECTS

Insect	Mortality			
	Control	Cry1C.563	Cry1C-R148A	Native Cry1C
<i>A. ipsilon</i>	—	—	—	—
<i>H. virescens</i>	—	+	+++	+
<i>H. zea</i>	—	—	—	—
<i>O. nubilalis</i>	—	+++	+++	++
<i>S. frugiperda</i>	—	+++	+++	+

+ = 20-49% mortality
++ = 50-74% mortality
+++ = 75-100% mortality

EG10368 transformants harboring random mutants at position R148 of Cry1C were evaluated in bioassay in a one-dose screen against *S. exigua* as described above. Five Cry1C mutants were identified with improved activity over wild-type Cry1C. The mutants were then evaluated in eight-dose bioassay against *S. exigua* as described above. All five Cry1C mutants gave a significantly lower LC₅₀ than wild-type Cry1C (Table 10), comparable to EG11822 (R148A). One mutant, designated EG11832 (Cry1C-R148D) gave a significantly lower LC₅₀ and LC₉₅ than EG11822, indicating further improved toxicity towards *S. exigua*.